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TITLE: **HYPERSENSITIVE RESPONSE ELICITING
DOMAINS AND USE THEREOF**

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HYPERSENSITIVE RESPONSE ELICITING DOMAINS AND USE THEREOF

This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 60/212,211, filed on June 16, 2000.

FIELD OF THE INVENTION

The present invention relates to hypersensitive response elicitors and
10 their structure.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular
bacterial growth, symptom development, and disease development in the host plant;
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a
particular type of incompatible interaction occurring, without progressive disease
symptoms. During compatible interactions on host plants, bacterial populations
20 increase dramatically and progressive symptoms occur. During incompatible
interactions, bacterial populations do not increase, and progressive symptoms do not
occur.

The hypersensitive response is a rapid, localized necrosis that is
associated with the active defense of plants against many pathogens (Kiraly, Z.,
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily
30 observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

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“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,

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et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention is a further advance in the effort to identify and characterize hypersensitive response elicitor proteins.

One aspect of the present invention is directed to an isolated
5 hypersensitive response elicitor protein comprising a pair of spaced apart domains,
with each comprising an acid portion linked to an alpha-helix.

10 Nucleic acid molecules encoding either of these proteins as well as
vectors, host cells, transgenic plants, and transgenic plant seeds containing those
nucleic acid molecules are also disclosed.

As an alternative to applying the protein to plants or plant seeds in order to impart disease resistance, to enhance plant growth, to control insects on plants, and/or impart stress resistance, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a nucleic acid molecule encoding the protein of the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or to impart stress resistance to the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the nucleic acid molecule encoding the protein of the present invention can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or to impart stress resistance to plants or plants grown from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing the construction of a universal expression cassette for a hypersensitive response domain.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to an isolated hypersensitive response elicitor protein comprising a pair of spaced apart domains, with each comprising an acid portion linked to an alpha-helix. The acidic portion is a polypeptide with 10 or more amino acids, is rich in acidic amino acids, and has a pI below 5.0. The acidic portion has a secondary structure in the form of a beta-sheet or a beta-turn. The secondary structure of this unit can also be in an unordered form.

The alpha-helix portion of the present invention is a polypeptide with 10 or more amino acids. Its secondary structure is in the form of a stable alpha-helix.

Another embodiment of the present invention relates to an isolated hypersensitive response elicitor protein comprising an acid portion linked to an alpha-helix.

Both of these proteins are capable of eliciting a hypersensitive response.

The alpha helix is a common structural motif of proteins in which a linear sequence of amino acid folds into a right-handed helix stabilized by internal hydrogen bonding between backbone atoms.

The acidic motif includes a certain combination of amino acids in which a linear sequence with a pI below 5.0 folds into a β sheet, coil, or thin structures but not an alpha helix of secondary structure.

The hypersensitive response elicitor polypeptides or proteins according to the present invention can be derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors

include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors
5 from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*,
10 *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

15	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
	1 5 10 15
	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
	20 25 30
20	Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
	35 40 45
	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
	50 55 60
	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
	65 70 75 80
25	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
	85 90 95
	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
	100 105 110
30	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
	115 120 125
	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
	130 135 140
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
	145 150 155 160
35	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
	165 170 175

	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	
				180					185					190			
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	
			195					200					205				
5	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	
		210					215					220					
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	
	225					230					235					240	
10	Gln	Tyr	Pro	Glu	Ile	Phe	Gly	Lys	Pro	Glu	Tyr	Gln	Lys	Asp	Gly	Trp	
					245					250					255		
	Ser	Ser	Pro	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	Lys	
				260					265					270			
	Pro	Asp	Asp	Asp	Gly	Met	Thr	Gly	Ala	Ser	Met	Asp	Lys	Phe	Arg	Gln	
			275					280					285				
15	Ala	Met	Gly	Met	Ile	Lys	Ser	Ala	Val	Ala	Gly	Asp	Thr	Gly	Asn	Thr	
		290					295					300					
	Asn	Leu	Asn	Leu	Arg	Gly	Ala	Gly	Gly	Ala	Ser	Leu	Gly	Ile	Asp	Ala	
	305					310				315						320	
20	Ala	Val	Val	Gly	Asp	Lys	Ile	Ala	Asn	Met	Ser	Leu	Gly	Lys	Leu	Ala	
					325				330						335		
	Asn	Ala															

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

30	CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTTCGA	CACCGTTACG	60
	GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
	GATCTGGTAT	TTCAGTTTGG	GGACACCGGG	CGTGAACCTCA	TGATGCAGAT	TCAGCCGGGG	180
	CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
	TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
35	CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAAC	GGCGGGAATG	360
	ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
	CGATCATTA	GATAAAGGCG	GCTTTTTTTA	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480

	CACCGTCGGC	GTCACCTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
	GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
	AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
	TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTTCCAGCG	TGGATAAACT	720
5	GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTTG	GCGGCGCGCT	780
	GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840
	TTTCGGCAAT	GGCGCGCAGG	GTGCGAGCAA	CCTGCTATCC	GTACCGAAAT	CCGGCGGCGA	900
	TGCGTTGTCA	AAAATGTTTG	ATAAAGCGCT	GGACGATCTG	CTGGGTCATG	ACACCGTGAC	960
	CAAGCTGACT	AACCAGAGCA	ACCAACTGGC	TAATTCAATG	CTGAACGCCA	GCCAGATGAC	1020
10	CCAGGGTAAT	ATGAATGCGT	TCGGCAGCGG	TGTGAACAAC	GCACTGTCTG	CCATTCTCGG	1080
	CAACGGTCTC	GGCCAGTCGA	TGAGTGGCTT	CTCTCAGCCT	TCTCTGGGGG	CAGGCGGCTT	1140
	GCAGGGCCTG	AGCGGCGCGG	GTGCATTCAA	CCAGTTGGGT	AATGCCATCG	GCATGGGCGT	1200
	GGGGCAGAA	GCTGCGCTGA	GTGCGTTGAG	TAACGTCAGC	ACCCACGTAG	ACGGTAACAA	1260
	CCGCCACTTT	GTAGATAAAG	AAGATCGCGG	CATGGCGAAA	GAGATCGGCC	AGTTTATGGA	1320
15	TCAGTATCCG	GAAATATTCG	GTAAACCGGA	ATACCAGAAA	GATGGCTGGA	GTTCGCCGAA	1380
	GACGGACGAC	AAATCCTGGG	CTAAAGCGCT	GAGTAAACCG	GATGATGACG	GTATGACCGG	1440
	CGCCAGCATG	GACAAATTCC	GTCAGGCGAT	GGGTATGATC	AAAAGCGCGG	TGGCGGGTGA	1500
	TACCGGCAAT	ACCAACCTGA	ACCTGCGTGG	CGCGGGCGGT	GCATCGCTGG	GTATCGATGC	1560
	GGCTGTCGTC	GGCGATAAAA	TAGCCAACAT	GTCGCTGGGT	AAGCTGGCCA	ACGCCTGATA	1620
20	ATCTGTGCTG	GCCTGATAAA	GCGGAAACGA	AAAAAGAGAC	GGGGAAGCCT	GTCTCTTTTC	1680
	TTATTATGCG	GTTTATGCGG	TTACCTGGAC	CGGTTAATCA	TCGTATCGCA	TCTGGTACAA	1740
	ACGCACATTT	TCCCGTTCAT	TCGCGTCGTT	ACGCGCCACA	ATCGCGATGG	CATCTTCCTC	1800
	GTCGCTCAGA	TTGCGCGGCT	GATGGGGAAC	GCCGGGTGGA	ATATAGAGAA	ACTCGCCGGC	1860
	CAGATGGAGA	CACGTCTGCG	ATAAATCTGT	GCCGTAACGT	GTTTCTATCC	GCCCCTTTAG	1920
25	CAGATAGATT	GCGGTTTCGT	AATCAACATG	GTAATGCGGT	TCCGCCTGTG	CGCCGGCCGG	1980
	GATCACCACA	ATATTCATAG	AAAGCTGTCT	TGCACCTACC	GTATCGCGGG	AGATACCGAC	2040
	AAAATAGGGC	AGTTTTTGCG	TGGTATCCGT	GGGGTGTTCC	GGCCTGACAA	TCTTGAGTTG	2100
	GTTTCGTCATC	ATCTTTCTCC	ATCTGGGCGA	CCTGATCGGT	T		2141

30 The hypersensitive response elicitor from *Erwinia chrysanthemi* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ.

ID. No. 1, from amino acid 69 to amino acid 122, particularly from amino acid 85 to amino acid 116. The acidic unit in the first domain extends, within SEQ. ID. No. 1, from amino acid 69 to amino acid 102, particularly from amino acid 85 to amino acid 102. The alpha-helix in the first domain extends, within SEQ. ID. No. 1, from amino acid 102 to amino acid 122, particularly from amino acid 102 to amino acid 116. The second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 299, particularly from amino acid 256 to amino acid 292. The acidic unit in the second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 279, particularly from amino acid 261 to amino acid 279. The alpha-helix in the second domain extends, within SEQ. ID. No. 1, from amino acid 279 to amino acid 299, particularly from amino acid 279 to amino acid 292.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser	
	1				5					10					15		
20	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln	
				20					25					30			
	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn	
			35					40					45				
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met	
		50					55					60					
25	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu	
	65					70					75					80	
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu	
				85					90					95			
30	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr	
				100					105					110			
	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro	
				115				120					125				
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser	
		130					135					140					
35	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln	
	145					150					155					160	

	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly	
					165					170					175		
	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu	
				180					185					190			
5	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly	
			195					200					205				
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly	
		210					215					220					
10	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu	
	225					230					235					240	
	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln	
					245					250					255		
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln	
				260					265					270			
15	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe	
			275					280					285				
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	
		290					295					300					
20	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro	
	305					310					315					320	
	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	
					325					330					335		
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn	
				340					345					350			
25	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn	
		355						360					365				
	Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp	
		370					375					380					
30	Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu	
	385					390					395					400	
	Gly	Ala	Ala														

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff,

D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5 AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA 60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT 120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG 180
10 GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG 240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG 300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA 360
GGACTGTCTGA ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA 420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC 480
15 TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC 540
CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG 600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC 660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG 720
CTCCTTGGCA ACGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC 780
20 GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG 840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT 900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCGATAC 1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC 1080
25 AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140
ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200
GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

30 The hypersensitive response elicitor from *Erwinia amylovora* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 74, particularly from amino acid 45 to amino

acid 68. The acidic unit in the first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 57, particularly from amino acid 45 to amino acid 57. The alpha-helix in the first domain extends, within SEQ. ID. No. 3, from amino acid 57 to amino acid 74, particularly from amino acid 57 to amino acid 68. The second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 180, particularly from amino acid 145 to amino acid 170. The acidic unit in the second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 157, particularly from amino acid 145 to amino acid 157. The alpha-helix in the second domain extends, within SEQ. ID. No. 3, from amino acid 157 to amino acid 180, particularly from amino acid 157 to amino acid 170.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

15 ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCTCGC CGGGTCTGTT CCAGTCCGGG 60
GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120
20 CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180
CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240
AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300
25 CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360
CAGGGCGGCG GGCAGATCGG CGATAATCCT TTA CTGAAAG CCATGCTGAA GCTTATTGCA 420
30 CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480
TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC 540
CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCGTCA GTACCTTCTC ACCCCCATCC 600
35 ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG 660
GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720
40 AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780
GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA 840
GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC 900
45 CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC 960
AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020
50 AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC 1080

AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140
 5 TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200
 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTAAAA GCGATAGCGA GGGGCTAAAC 1260
 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320
 10 GCCAACCTGA AGGTGGCTGA ATGA 1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 6 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
 1 5 10 15
 20 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
 20 20 25 30
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
 35 40 45
 25 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
 50 55 60
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
 65 70 75 80
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
 85 90 95
 35 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
 100 105 110
 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
 115 120 125
 40 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
 130 135 140
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
 145 150 155 160
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
 165 170 175
 50 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
 180 185 190
 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
 195 200 205
 55 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
 210 215 220

0927948-061201

	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225					230					235					240	
5	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
					245					250					255		
	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
10	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
			275					280					285				
	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
15			290				295					300					
	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
20					325					330					335		
	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
				340					345					350			
25	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	
			355					360					365				
	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	
30			370				375					380					
	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	
	385				390						395					400	
	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	
35				405						410					415		
	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	
			420						425					430			
40	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu		
			435					440					445				

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

This hypersensitive response elicitor from *Erwinia amylovora* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID. No. 6, from amino acid 5 to amino acid 64, particularly from amino acid 31 to amino acid 57. The acidic unit in the first domain extends, within SEQ. ID. No. 6, from amino acid 5 to amino acid 45, particularly from amino acid 31 to amino acid 45. The

alpha-helix in the first domain extends, within SEQ. ID. No. 6, from amino acid 45 to amino acid 64, particularly from amino acid 45 to amino acid 64. The second domain extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 146, particularly from amino acid 116 to amino acid 140. The acidic unit in the second domain
5 extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 131, particularly from amino acid 116 to amino acid 131. The alpha-helix in the second domain extends, within SEQ. ID. No. 6, from amino acid 131 to amino acid 146, particularly from amino acid 131 to amino acid 140.

Another potentially suitable hypersensitive response elicitor from
10 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

15 ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC 60
CCTGTGGGGC ATGGTGTTCG CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC 120
GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA 180
20 TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG 240
GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC 300
25 CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT 360
GAGGCGGCCG CGCCAGATGC GCGCGTCTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT 420
ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA 480
30 ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC 540
AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCAGTCGCC TGCAGCATTC ACCGCCGCAC 600
ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA 660
35 ACGGCCACAG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA 720
CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC 780
40 GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC 840
CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT 900
GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG 960
45 GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC 1020
TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC 1080
50 CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC 1140
GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA 1200

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	AGCGGCAAGA	TCTCGCTGGG	GAGCGGTACG	CAAAGTCACA	ACAAAACAAT	GCTAAGCCAA	1260
	CCGGGGGAAG	CGCACCGTTC	CTTATTAACC	GGCATTGTC	AGCATCCTGC	TGGCGCAGCG	1320
5	CGGCCGCAGG	GCGAGTCAAT	CCGCCTGCAT	GACGACAAAA	TTCATATCCT	GCATCCGGAG	1380
	CTGGGCGTAT	GGCAATCTGC	GGATAAAGAT	ACCCACAGCC	AGCTGTCTCG	CCAGGCAGAC	1440
10	GGTAAGCTCT	ATGCGCTGAA	AGACAACCGT	ACCTGCAAA	ACCTCTCCGA	TAATAAATCC	1500
	TCAGAAAAGC	TGGTCGATAA	AATCAAATCG	TATTCCGTTG	ATCAGCGGGG	GCAGGTGGCG	1560
	ATCCTGACGG	ATACTCCCGG	CCGCCATAAG	ATGAGTATTA	TGCCCTCGCT	GGATGCTTCC	1620
15	CCGGAGAGCC	ATATTTCCCT	CAGCCTGCAT	TTTGCCGATG	CCCACCAGGG	GTTATTGCAC	1680
	GGGAAGTCGG	AGCTTGAGGC	ACAATCTGTC	GCGATCAGCC	ATGGGCGACT	GGTTGTGGCC	1740
20	GATAGCGAAG	GCAAGCTGTT	TAGCGCCGCC	ATTCCGAAGC	AAGGGGATGG	AAACGAACTG	1800
	AAAATGAAAG	CCATGCCTCA	GCATGCGCTC	GATGAACATT	TGGTCATGA	CCACCAGATT	1860
	TCTGGATTTT	TCCATGACGA	CCACGGCCAG	CTTAATGCGC	TGGTGAAAAA	TAACCTCAGG	1920
25	CAGCAGCATG	CCTGCCCGTT	GGGTAACGAT	CATCAGTTTC	ACCCCGGCTG	GAACCTGACT	1980
	GATGCGCTGG	TTATCGACAA	TCAGCTGGGG	CTGCATCATA	CCAATCCTGA	ACCGCATGAG	2040
30	ATTCTTGATA	TGGGGCATTT	AGGCAGCCTG	GCGTTACAGG	AGGGCAAGCT	TCACTATTTT	2100
	GACCAGCTGA	CCAAAGGGTG	GACTGGCGCG	GAGTCAGATT	GTAAGCAGCT	GAAAAAAGGC	2160
	CTGGATGGAG	CAGCTTATCT	ACTGAAAGAC	GGTGAAGTGA	AACGCCTGAA	TATTAATCAG	2220
35	AGCACCTCCT	CTATCAAGCA	CGGAACGGAA	AACGTTTTTT	CGCTGCCGCA	TGTGCGCAAT	2280
	AAACCGGAGC	CGGGAGATGC	CCTGCAAGGG	CTGAATAAAG	ACGATAAGGC	CCAGGCCATG	2340
40	GCGGTGATTG	GGGTAAATAA	ATACCTGGCG	CTGACGAAA	AAGGGGACAT	TCGCTCCTTC	2400
	CAGATAAAAC	CCGGCACCCA	GCAGTTGGAG	CGGCCGGCAC	AAACTCTCAG	CCGCGAAGGT	2460
	ATCAGCGGCG	AACTGAAAGA	CATTGATGTC	GACCACAAGC	AGAACCTGTA	TGCCTTGACC	2520
45	CACGAGGGAG	AGGTGTTTCA	TCAGCCGCGT	GAAGCCTGGC	AGAATGGTGC	CGAAAGCAGC	2580
	AGCTGGCACA	AACTGGCGTT	GCCACAGAGT	GAAAGTAAGC	TAAAAAGTCT	GGACATGAGC	2640
50	CATGAGCACA	AACCGATTGC	CACCTTTGAA	GACGGTAGCC	AGCATCAGCT	GAAGGCTGGC	2700
	GGCTGGCAGC	CCTATGCGGC	ACCTGAACGC	GGGCCGCTGG	CGGTGGGTAC	CAGCGGTTCA	2760
	CAAACCGTCT	TTAACCGACT	AATGCAGGGG	GTGAAAGGCA	AGGTGATCCC	AGGCAGCGGG	2820
55	TTGACGGTTA	AGCTCTCGGC	TCAGACGGGG	GGAATGACCG	GCGCCGAAGG	GCGCAAGGTC	2880
	AGCAGTAAAT	TTTCCGAAAG	GATCCGCGCC	TATGCGTTCA	ACCCAACAAT	GTCCACGCCG	2940
60	CGACCGATTA	AAAATGCTGC	TTATGCCACA	CAGCACGGCT	GGCAGGGGCG	TGAGGGGTTG	3000
	AAGCCGTTGT	ACGAGATGCA	GGGAGCGCTG	ATTAAACAAC	TGGATGCGCA	TAACGTTTCG	3060
	CATAACGCGC	CACAGCCAGA	TTTGAGAGC	AAACTGGAAA	CTCTGGATTT	AGGCGAACAT	3120
65	GGCGCAGAAT	TGCTTAACGA	CATGAAGCGC	TTCCGCGACG	AACTGGAGCA	GAGTGCAACC	3180

65

CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTGCGATG 5160
GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220
5 CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCCTGTTAA ATCGGTCAGC 5280
GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340
10 AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400
CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460
CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

15

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.

ID. No. 8 as follows:

20

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
1 5 10 15

25

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
20 25 30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
35 40 45

30

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65 70 75 80

35

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
85 90 95

40

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
115 120 125

45

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145 150 155 160

50

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
180 185 190

55

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
195 200 205

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	Lys	Glu	Glu	Pro	Val	Gly	Ser	Thr	Ser	Lys	Ala	Thr	Thr	Ala	His	Ala
	210						215					220				
5	Asp	Arg	Val	Glu	Ile	Ala	Gln	Glu	Asp	Asp	Asp	Ser	Glu	Phe	Gln	Gln
	225					230				235					240	
	Leu	His	Gln	Gln	Arg	Leu	Ala	Arg	Glu	Arg	Glu	Asn	Pro	Pro	Gln	Pro
					245				250						255	
10	Pro	Lys	Leu	Gly	Val	Ala	Thr	Pro	Ile	Ser	Ala	Arg	Phe	Gln	Pro	Lys
				260				265					270			
	Leu	Thr	Ala	Val	Ala	Glu	Ser	Val	Leu	Glu	Gly	Thr	Asp	Thr	Thr	Gln
			275					280					285			
15	Ser	Pro	Leu	Lys	Pro	Gln	Ser	Met	Leu	Lys	Gly	Ser	Gly	Ala	Gly	Val
	290						295					300				
	Thr	Pro	Leu	Ala	Val	Thr	Leu	Asp	Lys	Gly	Lys	Leu	Gln	Leu	Ala	Pro
20	305					310					315					320
	Asp	Asn	Pro	Pro	Ala	Leu	Asn	Thr	Leu	Leu	Lys	Gln	Thr	Leu	Gly	Lys
					325					330					335	
25	Asp	Thr	Gln	His	Tyr	Leu	Ala	His	His	Ala	Ser	Ser	Asp	Gly	Ser	Gln
				340					345					350		
	His	Leu	Leu	Leu	Asp	Asn	Lys	Gly	His	Leu	Phe	Asp	Ile	Lys	Ser	Thr
		355						360					365			
30	Ala	Thr	Ser	Tyr	Ser	Val	Leu	His	Asn	Ser	His	Pro	Gly	Glu	Ile	Lys
		370					375					380				
	Gly	Lys	Leu	Ala	Gln	Ala	Gly	Thr	Gly	Ser	Val	Ser	Val	Asp	Gly	Lys
35	385					390					395				400	
	Ser	Gly	Lys	Ile	Ser	Leu	Gly	Ser	Gly	Thr	Gln	Ser	His	Asn	Lys	Thr
				405						410				415		
40	Met	Leu	Ser	Gln	Pro	Gly	Glu	Ala	His	Arg	Ser	Leu	Leu	Thr	Gly	Ile
			420						425					430		
	Trp	Gln	His	Pro	Ala	Gly	Ala	Ala	Arg	Pro	Gln	Gly	Glu	Ser	Ile	Arg
		435					440						445			
45	Leu	His	Asp	Asp	Lys	Ile	His	Ile	Leu	His	Pro	Glu	Leu	Gly	Val	Trp
	450						455					460				
	Gln	Ser	Ala	Asp	Lys	Asp	Thr	His	Ser	Gln	Leu	Ser	Arg	Gln	Ala	Asp
50	465					470					475					480
	Gly	Lys	Leu	Tyr	Ala	Leu	Lys	Asp	Asn	Arg	Thr	Leu	Gln	Asn	Leu	Ser
				485						490				495		
55	Asp	Asn	Lys	Ser	Ser	Glu	Lys	Leu	Val	Asp	Lys	Ile	Lys	Ser	Tyr	Ser
				500					505					510		
	Val	Asp	Gln	Arg	Gly	Gln	Val	Ala	Ile	Leu	Thr	Asp	Thr	Pro	Gly	Arg
			515					520					525			
60	His	Lys	Met	Ser	Ile	Met	Pro	Ser	Leu	Asp	Ala	Ser	Pro	Glu	Ser	His
	530						535					540				
	Ile	Ser	Leu	Ser	Leu	His	Phe	Ala	Asp	Ala	His	Gln	Gly	Leu	Leu	His
65	545					550					555					560

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	Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg	565	570	575
5	Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro	580	585	590
	Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His	595	600	605
10	Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe	610	615	620
	His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg	625	630	635
15	Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly	645	650	655
	Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His	660	665	670
	His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly	675	680	685
25	Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr	690	695	700
	Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly	705	710	715
30	Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu	725	730	735
	Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val	740	745	750
35	Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu	755	760	765
40	Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly	770	775	780
	Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe	785	790	795
45	Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu	805	810	815
	Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His	820	825	830
	Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln	835	840	845
55	Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys	850	855	860
	Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser	865	870	875
60	His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln	885	890	895

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	Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro	
				900					905					910			
5	Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met	
			915				920						925				
	Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys	
		930				935						940					
10	Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val	
	945				950					955						960	
	Ser	Ser	Lys	Phe	Ser	Glu	Arg	Ile	Arg	Ala	Tyr	Ala	Phe	Asn	Pro	Thr	
15				965					970					975			
	Met	Ser	Thr	Pro	Arg	Pro	Ile	Lys	Asn	Ala	Ala	Tyr	Ala	Thr	Gln	His	
				980					985					990			
20	Gly	Trp	Gln	Gly	Arg	Glu	Gly	Leu	Lys	Pro	Leu	Tyr	Glu	Met	Gln	Gly	
		995					1000						1005				
	Ala	Leu	Ile	Lys	Gln	Leu	Asp	Ala	His	Asn	Val	Arg	His	Asn	Ala	Pro	
		1010					1015					1020					
25	Gln	Pro	Asp	Leu	Gln	Ser	Lys	Leu	Glu	Thr	Leu	Asp	Leu	Gly	Glu	His	
	1025				1030						1035					1040	
	Gly	Ala	Glu	Leu	Leu	Asn	Asp	Met	Lys	Arg	Phe	Arg	Asp	Glu	Leu	Glu	
30				1045					1050					1055			
	Gln	Ser	Ala	Thr	Arg	Ser	Val	Thr	Val	Leu	Gly	Gln	His	Gln	Gly	Val	
				1060					1065					1070			
35	Leu	Lys	Ser	Asn	Gly	Glu	Ile	Asn	Ser	Glu	Phe	Lys	Pro	Ser	Pro	Gly	
		1075					1080						1085				
	Lys	Ala	Leu	Val	Gln	Ser	Phe	Asn	Val	Asn	Arg	Ser	Gly	Gln	Asp	Leu	
		1090				1095					1100						
40	Ser	Lys	Ser	Leu	Gln	Gln	Ala	Val	His	Ala	Thr	Pro	Pro	Ser	Ala	Glu	
	1105				1110					1115						1120	
	Ser	Lys	Leu	Gln	Ser	Met	Leu	Gly	His	Phe	Val	Ser	Ala	Gly	Val	Asp	
45				1125					1130					1135			
	Met	Ser	His	Gln	Lys	Gly	Glu	Ile	Pro	Leu	Gly	Arg	Gln	Arg	Asp	Pro	
				1140				1145					1150				
50	Asn	Asp	Lys	Thr	Ala	Leu	Thr	Lys	Ser	Arg	Leu	Ile	Leu	Asp	Thr	Val	
		1155					1160						1165				
	Thr	Ile	Gly	Glu	Leu	His	Glu	Leu	Ala	Asp	Lys	Ala	Lys	Leu	Val	Ser	
		1170				1175					1180						
55	Asp	His	Lys	Pro	Asp	Ala	Asp	Gln	Ile	Lys	Gln	Leu	Arg	Gln	Gln	Phe	
	1185				1190					1195						1200	
	Asp	Thr	Leu	Arg	Glu	Lys	Arg	Tyr	Glu	Ser	Asn	Pro	Val	Lys	His	Tyr	
60				1205					1210					1215			
	Thr	Asp	Met	Gly	Phe	Thr	His	Asn	Lys	Ala	Leu	Glu	Ala	Asn	Tyr	Asp	
		1220						1225					1230				
65	Ala	Val	Lys	Ala	Phe	Ile	Asn	Ala	Phe	Lys	Lys	Glu	His	His	Gly	Val	
		1235					1240						1245				

0907948 "061001

	Asn	Leu	Thr	Thr	Arg	Thr	Val	Leu	Glu	Ser	Gln	Gly	Ser	Ala	Glu	Leu	
	1250						1255					1260					
5	Ala	Lys	Lys	Leu	Lys	Asn	Thr	Leu	Leu	Ser	Leu	Asp	Ser	Gly	Glu	Ser	
	1265					1270					1275					1280	
	Met	Ser	Phe	Ser	Arg	Ser	Tyr	Gly	Gly	Gly	Val	Ser	Thr	Val	Phe	Val	
10					1285					1290					1295		
	Pro	Thr	Leu	Ser	Lys	Lys	Val	Pro	Val	Pro	Val	Ile	Pro	Gly	Ala	Gly	
				1300					1305					1310			
15	Ile	Thr	Leu	Asp	Arg	Ala	Tyr	Asn	Leu	Ser	Phe	Ser	Arg	Thr	Ser	Gly	
			1315					1320					1325				
	Gly	Leu	Asn	Val	Ser	Phe	Gly	Arg	Asp	Gly	Gly	Val	Ser	Gly	Asn	Ile	
	1330					1335					1340						
20	Met	Val	Ala	Thr	Gly	His	Asp	Val	Met	Pro	Tyr	Met	Thr	Gly	Lys	Lys	
	1345				1350					1355					1360		
	Thr	Ser	Ala	Gly	Asn	Ala	Ser	Asp	Trp	Leu	Ser	Ala	Lys	His	Lys	Ile	
25					1365					1370					1375		
	Ser	Pro	Asp	Leu	Arg	Ile	Gly	Ala	Ala	Val	Ser	Gly	Thr	Leu	Gln	Gly	
				1380					1385					1390			
30	Thr	Leu	Gln	Asn	Ser	Leu	Lys	Phe	Lys	Leu	Thr	Glu	Asp	Glu	Leu	Pro	
			1395				1400					1405					
	Gly	Phe	Ile	His	Gly	Leu	Thr	His	Gly	Thr	Leu	Thr	Pro	Ala	Glu	Leu	
	1410				1415					1420							
35	Leu	Gln	Lys	Gly	Ile	Glu	His	Gln	Met	Lys	Gln	Gly	Ser	Lys	Leu	Thr	
	1425				1430					1435					1440		
	Phe	Ser	Val	Asp	Thr	Ser	Ala	Asn	Leu	Asp	Leu	Arg	Ala	Gly	Ile	Asn	
40					1445					1450					1455		
	Leu	Asn	Glu	Asp	Gly	Ser	Lys	Pro	Asn	Gly	Val	Thr	Ala	Arg	Val	Ser	
			1460					1465					1470				
45	Ala	Gly	Leu	Ser	Ala	Ser	Ala	Asn	Leu	Ala	Ala	Gly	Ser	Arg	Glu	Arg	
	1475				1480					1485							
	Ser	Thr	Thr	Ser	Gly	Gln	Phe	Gly	Ser	Thr	Thr	Ser	Ala	Ser	Asn	Asn	
	1490				1495					1500							
50	Arg	Pro	Thr	Phe	Leu	Asn	Gly	Val	Gly	Ala	Gly	Ala	Asn	Leu	Thr	Ala	
	1505				1510					1515					1520		
	Ala	Leu	Gly	Val	Ala	His	Ser	Ser	Thr	His	Glu	Gly	Lys	Pro	Val	Gly	
55					1525					1530					1535		
	Ile	Phe	Pro	Ala	Phe	Thr	Ser	Thr	Asn	Val	Ser	Ala	Ala	Leu	Ala	Leu	
			1540					1545					1550				
60	Asp	Asn	Arg	Thr	Ser	Gln	Ser	Ile	Ser	Leu	Glu	Leu	Lys	Arg	Ala	Glu	
	1555				1560					1565							
	Pro	Val	Thr	Ser	Asn	Asp	Ile	Ser	Glu	Leu	Thr	Ser	Thr	Leu	Gly	Lys	
	1570				1575					1580							

09079248 "061201

His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
 1585 1590 1595 1600
 5 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
 1605 1610 1615
 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
 1620 1625 1630
 10 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
 1635 1640 1645
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
 1650 1655 1660
 15 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
 1665 1670 1675 1680
 20 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
 1685 1690 1695
 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
 1700 1705 1710
 25 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
 1715 1720 1725
 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
 1730 1735 1740
 30 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
 1745 1750 1755 1760
 35 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
 1765 1770 1775
 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
 1780 1785 1790
 40 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
 1795 1800 1805
 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
 1810 1815 1820
 45 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
 1825 1830 1835

50 This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

55 ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG 60
 ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120
 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180
 60 GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240

GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT 300
 CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC 360
 5 GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
 1 5 10 15
 15 Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
 20 25 30
 Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
 35 40 45
 20 Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
 50 55 60
 25 Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80
 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95
 30 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110
 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125
 35 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
 130 135

40 This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

45 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1 5 10 15
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
 20 25 30
 50 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60

	Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val	
	65					70				75					80		
	Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe	
				85						90					95		
5	Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met	
				100					105					110			
	Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu	
			115					120					125				
10	Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met	
		130					135					140					
	Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro	
	145					150					155					160	
	Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe	
					165					170					175		
15	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile	
				180					185					190			
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	
			195					200					205				
20	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	
		210					215						220				
	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	
		225				230					235					240	
	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	
				245						250					255		
25	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	
			260						265					270			
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	
			275					280					285				
30	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala	
		290					295					300					
	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala	
		305				310					315					320	
	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg	
				325						330					335		
35	Asn	Gln	Ala	Ala	Ala												
				340													

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

Further information about the hypersensitive response elicitor derived from

Pseudomonas syringae is found in He, S. Y., H. C. Huang, and A. Collmer,

- 5 “*Pseudomonas syringae* pv. *syringae* Harpin_{PSS}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

10 ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTTG 60
GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120
GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180
AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240
15 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480
AAGCCGGA CT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540
20 GAAACGGCTG CGTTCCGTTT GGCACCTGAC ATCATTTGCC AGCAACTGGG TAATCAGCAG 600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTC 660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG 840
25 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020
GCCTGA 1026

- 30 Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,

which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

5	TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG	60
	CTGAGTGCGC AGATTTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTC AAGG	120
	CCTCTGAGTG CCGTGCGGAG CAATACCAGT CTTCTGCTG GCGTGTGCAC ACTGAGTTCG	180
10	AGGCATAGGC ATTTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA	240
	ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG	300
15	AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC	360
	TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCGCAT	420
	GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCAG CCACTCGATT TTTCGGCGCT	480
20	AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA	540
	CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA	600
25	CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC	660
	TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA	720
	GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGCGGGGCT	780
30	CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG	840
	CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG	900
35	TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC	960
	CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACGCAA ATCACTCCGC AGTTGGCCAA	1020
	CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTCTA CCGAGCAAGC	1080
40	CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGTCCGGC GCTGGCGAAG TCTTTGACGG	1140
	CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA	1200
45	GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA	1260
	CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT	1320
	GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT	1380
50	CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT	1440
	CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT	1500
55	TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC	1560
	TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG	1620
	CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA	1680
60	CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC	1729

This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

5	Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu	1 5 10 15
10	Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly	20 25 30
	Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly	35 40 45
15	Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val	50 55 60
20	Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile	65 70 75 80
	Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr	85 90 95
25	Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln	100 105 110
	Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser	115 120 125
30	Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr	130 135 140
35	Pro Ser Ala Thr Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly	145 150 155 160
	Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly	165 170 175
40	Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr	180 185 190
	Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr	195 200 205
45	Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile	210 215 220
50	Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp	225 230 235 240
	Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp	245 250 255
55	Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr	260 265 270

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Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
 275 280 285
 5 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
 290 295 300
 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
 305 310 315 320
 10 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
 325 330 335
 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
 340 345 350
 15 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
 355 360 365
 20 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
 370 375 380
 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
 385 390 395 400
 25 Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
 405 410 415
 30 Ala Ser Thr Gln His Thr Glu Leu
 420

This protein or polypeptide is about 42.9 kDa.

This hypersensitive response elicitor from *Pseudomonas syringae* has 1
 35 hypersensitive response eliciting domain. This domain extends, within SEQ. ID. No.
 14, from amino acid 45 to amino acid 102, particularly from amino acid 58 to amino
 acid 92. The acidic unit in the first domain extends, within SEQ. ID. No. 14, from
 amino acid 45 to amino acid 79, particularly from amino acid 58 to amino acid 79.
 The alpha-helix in the first domain extends, within SEQ. ID. No. 14, from amino acid
 40 79 to amino acid 102, particularly from amino acid 79 to amino acid 92.

The hypersensitive response elicitor polypeptide or protein derived
 from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.
 ID. No. 15 as follows:

45 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
 20 25 30

	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile	
			35					40					45				
	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly	
		50					55					60					
5	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	
	65					70					75				80		
	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	
					85					90					95		
10	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
15	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155				160		
	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
					165					170					175		
20	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
				180					185					190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
			195					200					205				
	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
		210					215					220					
25	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	
	225					230					235				240		
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	
					245					250					255		
30	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	
				260					265					270			
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	
			275					280					285				
	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	
		290					295					300					
35	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val	
	305					310					315				320		
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln	
					325					330					335		

09879248 "061201

Gln Ser Thr Ser Thr Gln Pro Met
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 16 as follows:

```

5  ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC      60
   AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC      120
   GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC      180
   GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC      240
   AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC      300
10 GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA      360
   GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG      420
   GGCAACGCGG TGGGCGGTGC CAACGCGGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC      480
   GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC      540
   GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT      600
15 GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC      660
   GGCCCGCAGA ACGCAGGCGA TGTCACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC      720
   CAGGGCGGCC TCACCGCGCT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG      780
   ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC      840
   GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT      900
20 GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC      960
   GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG      1020
   ACGCAGCCGA TGTAAT                                     1035

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25 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994),

30 which is hereby incorporated by reference.

The hypersensitive response elicitor from *Pseudomonas solanacearum* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID.

No. 15, from amino acid 85 to amino acid 131, particularly from amino acid 95 to amino acid 123. The acidic unit in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 123. The alpha-helix in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 111. The second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 264, particularly from amino acid 229 to amino acid 258. The acidic unit in the second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 246, particularly from amino acid 229 to amino acid 264. The alpha-helix in the second domain extends, within SEQ. ID. No. 15, from amino acid 246 to amino acid 264, particularly from amino acid 246 to amino acid 258.

The N-terminus of the hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

Met Asp Gly Ile Gly Asn His Phe Ser Asn
1 5 10

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not

Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” 8th Int’l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., “Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

- 5 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., “Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,”
- 10 Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., “Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco,” Eur. J. Biochem., 183:555-63 (1989), Ricci et al., “Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*,” Plant Path. 41:298-307 (1992),
- 15 Baillreul et al, “A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance,” Plant J., 8(4):551-60 (1995), and Bonnet et al., “Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants,” Eur. J. Plant Path., 102:181-92 (1996), which are hereby
- 20 incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

- 25 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

- 30 Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide
5 that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the
10 elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular
15 portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and
20 pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal
25 fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168,
30 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of

SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA. Suitable stringency conditions also include hybridization in a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C where hybridized nucleic acids remain bound when subject to washing the SSC buffer at a temperature of 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of 42°C where hybridized nucleic acids remain bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

A particularly advantageous aspect of the present invention involves utilizing a protein having a pair or more, particularly 3 or more, coupled domains. These domains can be from different source organisms. When a DNA molecule encoding such a protein is prepared, it can be advantageously used to make transgenic plants. The use of a gene encoding such domains, as opposed to a gene encoding a full length hypersensitive response elicitor, has a number of benefits. Firstly, such a gene is easier to synthesize. More significantly, the use of a plurality of domains together from different source organisms can impart their combined benefits to a transgenic plant.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant

DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria

Promoters vary in their “strength” (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its

bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, plant cells as well as

prokaryotic and eukaryotic cells, such as bacteria, virus, yeast, mammalian, insect cells, and the like.

5 The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance to plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to
10 impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart stress resistance.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance to
15 the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to
20 control insects, and/or to impart stress resistance. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or
25 to impart stress resistance.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat,
30 barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash,

pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting
10 disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

 The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of
15 pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas*
20 *campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

 With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant
25 growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit
30 and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their

growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any
5 form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants,
10 preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on
15 over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm),
20 pepper maggot, and tomato pinworm. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

Another aspect of the present invention is directed to imparting stress resistance to plants. Stress encompasses any environmental factor having an adverse
25 effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy
30 metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.

Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g.,

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dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies.

- 5 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

- The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are
10 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

- Another method of introducing the DNA molecule into plant cells is to
15 infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration
20 medium without antibiotics at 25-28°C.

- Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized
25 only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

- Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A.*
30 *rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, control of insects on the plant, and/or stress resistance. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance. While not

wishing to be bound by theory, such disease resistance, growth enhancement, insect control, and/or stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Bacterial Strains and Plasmids

Escherichia coli DH5 and BL21 were purchased from Gibco BRL (Rockville, MD) and Novagen (Madison, WI) respectively. pET28 plasmids were from Novagen (Madison, WI). All restriction enzymes (e.g., NdeI and HindIII), T4 DNA ligase, Calf intestinal alkaline phosphatase (CIP), and PCR reagents were from Gibco BRL (Rockville, MD). Oligonucleotides were synthesized by Lofstrand Labs Ltd (Gaithersburg, MD). Chemically synthesized polypeptides were synthesized by Bio-Synthesis (Lewisville, TX).

Example 2 - Construction of Truncated Gene Encoding Harpin

Fragments of genes encoding harpin proteins were constructed in pET28 vector and expressed in *E. coli* as follows;

1. HrpN fragments were PCR amplified from the pCPP2139 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
2. HrpZ fragments were PCR amplified from the pSYH10 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
3. PopA fragments were PCR amplified from the pBS::popA plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
4. HrpW fragments were PCR amplified from the pCPP1233 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.

All truncated fragments were amplified by PCR with full length harpin DNA as the template.

Oligonucleotides corresponding to the truncated N-terminal sequence were started /modified with a Nde I site (which serves as an initiation codon of methionine (ATG)). Oligonucleotides corresponding to a C-terminal sequence contained a UAA stop codon followed by a Hind III site.

PCR was carried in a 0.5 ml tube with GeneAmp™ 9600 and 9700 (PE Applied Biosystems, Branchburg, New Jersey). 45 µl of SuperMix™ (Gibco BRL, Rockville, MD) was mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and diH₂O to fill the final volume to 50 µl. After heating the mixture at 95°C for 2 min., PCR was performed for 30 cycles at 94°C for 1 min., 58°C for 1 min. and 72°C for 1.5 min. Amplified DNAs were purified with QIAquick PCR purification kit (QIAGEN Inc., Vlenica, CA), digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:24:1), and precipitated with ethanol. 5 µg of pET28(b) vector DNA was digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with calf intestinal alkaline phosphatase treatment for 30 min. at 37°C to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried at 14°C for 12 hours in a 15 µl mixture containing about 50 to

100 ng of digested pET28(b), 10 to 30 ng of targeted PCR fragments, and 1 unit of T4 DNA ligase. 5 µl of ligation solution was added to 100 µl of DH5α/XL1-Blue competent cells, placed in 15 ml Falcon tube, and incubated on ice for 30 min. After heat shock at 42°C for 45 seconds, 0.9 ml SOC solution (20 g bacto-tryptone, 5 g bacto-yeast extracts, 0.5 g NaCl, 20 mM glucose in one liter) was added into the tube and incubated at 37°C for 1 hour. 20 µl of transformed cells were plated onto LB agar plate with 30 µg/ml of kanamycin and incubated at 37°C for 14 hours. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared in a 2 ml culture with QIAprep Miniprep kit according to the manufacture's instruction. The DNA sequence of truncated harpin constructions was verified with restriction enzyme analysis and sequencing analysis. Plasmids with the desired DNA sequence were transferred into the BL21 strain with a standard chemical transformation method as indicated above.

15 **Example 3 - Expression of Proteins**

A single clone of *E. coli* with a constructed gene was grown overnight at 37°C in LB with kanamycin. A proper amount of overnight culture was transferred to 50 to 500 ml LB and incubated at 37°C until OD600 reached 0.5 to 0.8. ITPG was added to the culture which was further incubated at room temperature for a period of 5 hour to overnight. Alternatively, a proper amount of overnight culture was transferred to 50 to 500 ml of ½ TB with lactose medium (6 g bacto-trypton, 12 g bacto-yeast extract, 75 g lactose in one liter). After incubation at 37°C until the OD600 reached 0.5 to 0.8, the culture was incubated at room temperature for a period of 5 hours to overnight.

All bacterial cells were harvested by centrifugation and resuspended in 1:5 TE buffer (10 mM Tris, pH 8.5 and 1 mM EDTA). The cells were disrupted by sonication and clarified by centrifugation. Supernatants were then infiltrated into tobacco leaves for HR testing.

Heat treatment (i.e. boiling for 1 to 10 min.) was used to achieve further purification.

All truncated fragments of genes encoding harpin protein were expressed in *E. coli*/ BL-21, DE3 strain with an N-terminal His-tag and 20 to 21

amino acid residues generated from the expression vector sequence. The His-tag sequence did not affect the HR activity of the proteins. In some cases, Ni-Agarose beads were added into supernatant solution and mixed at 4°C to room temperature for a period of 30 min. to overnight. The proteins bound to the Ni-Agarose beads were washed by 0.1 M imidazole buffer, and proteins were eluted with 0.6 to 1.0 M imidazole. After dialysis against 10 mM Tris, pH 8.5 buffer, the proteins were infiltrated into tobacco leaves for HR testing.

For proteins expressed in *E. coli* that were difficult to dissolve in water, total cells were resuspended and sonicated in 8 M urea buffer (0.1M Na-phosphate, 10 mM Tris buffer, pH8.0). The total cell lysate was centrifuged, and supernatants were collected. Ni-agarose was added into the supernatants and mixed gently at room temperature for 30 min. The Ni-agarose resin was washed with buffer (8 M urea, 0.1 M Na-phosphate, 10 mM Tris buffer, pH6.3). The proteins were eluted with elution buffer (8 M urea, 0.1 M EDTA, 0.1 M Na-phosphate, 10 mM Tris buffer, pH 6.3) and dialyzed against buffer (pH 8.5, 10 mM Tris) with stepwise decreased urea. If the proteins still were insoluble in buffer, the solution pH was adjusted to 9 to 11 and sonicated at room temperature for 1 to 5 min.

Chemically synthesized polypeptides were dissolved in 10 mM Tris, pH 6.5 to 11 buffers depending on their solubility.

A hypersensitive response (“HR”) assay was performed by infiltration of 0.1 to 0.3 ml of serial diluted protein solutions into tobacco leaves (cv. Xanth). All HR data shown in these examples were recorded from 48 hours after infiltration.

Example 4 - Quantification of Proteins

All expressed proteins were checked with pre-cast 4-20% SDS polyacrylamide gel electrophoresis (SDS-PAGE) from Novex (San Diego, CA). After electrophoresis, the gel was stained with Coomassie R-250 solution (0.1% Coomassie R-250, 10% Acetate Acid, 40% ethanol) for 1 to 4 hours and destained with destaining solution (8% acetate acid and 25% ethanol) overnight. The density of corresponding bands were compared to standard proteins, which were either purchased from Novex or were from quantitative standard harpin protein produced by Eden Bioscience (Bothell, Washington).

Example 5 - Classification of Harpin Proteins

Since harpin proteins share common biochemical and biophysical characteristics as well as biological functions, based on their unique properties, HR elicitors from various pathogenic bacteria should be viewed as belonging to a new protein family—i.e. the harpin protein family. The harpin protein can be classified into at least four subfamilies based on their primary structure and isolated sources. As set forth in Table 1, those subfamilies are identified by the designation N, W, Z, A, etc.

Table 1 - Subfamilies of Harpin Proteins

Harpin proteins	Isolated Source	Classified Subfamily	pI	Amino acids	Heat stable	Core structure
HrpN _{Ea}	<i>E. amylovora</i>	N	4.42	403	Yes	No
HrpN _{Ech}	<i>E. chrysanthemi</i>	N	6.51	340	Yes	No
HrpN _{Ecc}	<i>E. carotovora</i>	N	5.82	356	Yes	No
HrpN _{Est}	<i>E. stewartii</i>	N	N/A	N/A	Yes	No
HrpW _{Pss}	<i>P. syringae</i>	W	4.43	424	Yes	No
HrpW _{Ea}	<i>E. amylovora</i>	W	4.46	447	Yes	No
HrpZ _{Pss}	<i>P. syringae</i>	Z	3.95	341	Yes	No
PopA1	<i>R. solanacearum</i>	A	4.16	344	Yes	No

Example 6 - Analysis of the Structural Units of an HR Domain

The sequence of amino acids that alone could elicit a hypersensitive response in plants (i.e. HR domains) has been investigated in different ways. It was reported that a carboxyl-terminal 148 amino acid portion of HrpZ_{Pss} is sufficient and necessary for HR (He et al., “Pseudomonas Syringae pv. Syringae Harpin_{pss}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” *Cell* 73:1255-1266.(1993), which is hereby incorporated by reference). With truncated HrpZ fragments, it was determined that an N-terminal 109 amino acids and C-terminal 216 amino acids of HrpZ_{Pss}, respectively, were found to elicit HR (Alfano et al., “Analysis of the Role of the Pseudomonas Syringae pv. Syringae HrpZ Harpin in Elicitation of the Hypersensitive Response in Tobacco Using

Functionally Non-polar hrpZ Deletion Mutations, Truncated HrpZ Fragments, and hrmA Mutations,” Molecular Microbiology 19:715-728 (1996), which is hereby incorporated by reference). Jin et al., “A Truncated Fragment of Harpin_{pss} Induces Systemic Resistance to *Xanthomonas campestris* pv. *Oryzae* in Rice,” Physiological and Molecular Plant Pathology 51:243-257 (1997), which is hereby incorporated by reference, reported that a truncated HrpZ_{pss} with an N-terminal of 137 amino acids elicited a hypersensitive response in tobacco and induced systemic acquired resistance (i.e. SAR) in rice. After digestion with protease, a hypersensitive response active fragment of HrpN_{Ea} was isolated and found to span amino acids 137 to 204 of HrpN_{Ea}. It was found that a 98 residue of N-terminal HrpN_{Ea} fragment was the smallest bacterially produced peptide that displayed HR-eliciting activity (Laby, “Molecular Studies on Interactions Between *Erwinia Amylovora* and its Host and Non-host Plants,” Doctoral Thesis in Cornell University (1997), which is hereby incorporated by reference).

A series of HrpN_{Ea} fragments have been generated with His-tag fusion at the N-terminal of the polypeptides and a polypeptide (HrpN_{Ea}137180), located at position of 137 to 180 amino acid residue of HrpN_{Ea}, was identified to elicit HR activity in tobacco.

Example 7 - Analysis of Secondary Structure of HR Domains

The DNA and primary protein sequence of the HrpN_{Ea}137180 show no any homologues among other hypersensitive response elicitors.

Analyses of the secondary structure of the fragment of HrpN_{Ea}137180 revealed, with the aid of the computer program Clone Manger5 (Scientific & Educational Software, Durham, NC), that there was a beta-form, a beta-turn, and unordered forms. One typical α -helical segment of residues at 157-170 was found in the HrpN_{Ea}137180 polypeptide. To determine the function of this structure, polypeptides with a disrupted α -helical structure were generated and hypersensitive response results were evaluated. As shown in Table 2, a complete alpha-helix unit (H unit), probably with a length greater than 12 amino acid residues, is need for hypersensitive response activity.

Table 2 - Effect of Alpha-helix Structure

Fragment name	Amino acid	HR*	Structure	Source
HrpN _{Ea} 137180	137-180 (44) pI= 3.10	+ <5 µg/ml	Complete H	E.coli expressed peptide
HrpN _{Ea} 137166	137-166 (30) pI = 3.29	-	disrupted H	Synthesized peptide
HrpN _{Ea} 76168	76-168 pI = 3.39	-	disrupted H	E.coli expressed peptide

5 The α -helical unit plays an important role in hypersensitive response activity; however, it was found that an α -helix unit alone did not achieve HR (Table 3).

10 Therefore, hypersensitive response eliciting domains contain more than one structure unit. Besides the core α -helical unit, there is an acidic unit that has no typical secondary structure feature but is rich in acidic amino acids. This relaxed structure, having a sheet and random turn, is designated as an acidic unit (A unit).

 Although the acidic unit is important in achieving a hypersensitive response, it alone, like the α -helical unit alone, did not elicit a hypersensitive response.

15 A synthetic polypeptide, HrpN_{Ea}140176, that included both A and H structure, spanning amino acids 140 to 176 of HrpN_{Ea}, gave full activity of HR. Sequence analysis by major search engines revealed no global primary sequence similarity in the databases to HrpN_{Ea}140176, even among the harpin protein families.

20 **Table 3 - Effect of Acidic Unit on Hypersensitive Response (HR) Activity**

Fragment name	Amino acid	HR*	Structure (A or H)**	Source
HrpN _{Ea} 140176	140-176 (37) pI=3.17	+ <5 µg/ml	A + H	Synthesized peptide
HrpN _{Ea} 157170	157-170 (14) pI = 6.94	-	H	Synthesized peptide
HrpN _{Ea} 137156	137-156 (20) pI = 2.67	-	A	Synthesized peptide

Example 8 - Hypersensitive Response Domain Structure of HrpN_{Ea}

Four α -helical regions with at least 12 amino acid residues were found in HrpN_{Ea} based on computer analysis with the program Clone Manager 5 (Scientific & Educational Software, Durham, NC), which predicts the secondary structure of protein from the primary sequence by the method of Garnier-Osguthorpe-Robson.

It is believed that a hypersensitive response domain includes two structural units, the α -helix (H) and the acidic unit (A). Another hypersensitive response domain, spanning amino acids 43 to 70 in HrpN_{Ea}, was found. A minimal sequence of 12 to 14 AA residues of both the H and A units is believed to be needed. The chemically synthesized polypeptide of HrpN_{Ea}4370 gave full HR activity in tobacco. Thus, a second HR domain has been discovered based on purely secondary structure analysis and prediction.

To further test the hypothesis that the A and H units are needed to achieve a hypersensitive response, an approach of unit exchange (i.e. swapping an acidic unit from one HR domain to another HR domain) was designed. A polypeptide of HrpN_{Ea}Dswap, which consisted of the acidic unit of a hypersensitive response domain (HrpN_{Ea}140176), spanning amino acids 136 to 156 of HrpN_{Ea}, and the α -helical unit of another hypersensitive response domain (HrpN_{Ea}4370), spanning amino acids 57 to 70 of HrpN_{Ea}, was chemically synthesized. This polypeptide swapped two structural units of A and H between two hypersensitive response domains of HrpN_{Ea}4370 and HrpN_{Ea}140176. The HrpN_{Ea}Dswap gave a hypersensitive response activity in tobacco (Table 4). This result shows that the structural characteristic of an HR domain determines its activity, and structural analysis can be used to determine hypersensitive response activity.

Table 4 - Two Structural Units Determine Hypersensitive Response Activity

Fragment name	Amino acid	HR	Structure Type	Source
HrpN _{Ea} 4370	43-70 (28) pI= 3.09	+ <5 μ g/ml	A + H	Synthesized peptide Partial soluble
HrpN _{Ea} Dswap	HrpN136156 (A)+ HrpN5770 (H) pI=2.67	<20 μ g/ml	A unit from HrpN _{Ea} 140176 + H unit from HrpN _{Ea} 4370	Synthesized peptide Partial soluble

Example 9 - Prediction of Hypersensitive Response Domains Among Proteins in Harpin Family

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The secondary structure which indicates the presence of a hypersensitive response domain in HrpNEa was used to identify other harpin proteins, including proteins classified as different subfamilies. Structural prediction of a hypersensitive response domain among harpin proteins was carried according to

10 following criteria:

1. There are two structural units in a hypersensitive response domain, including:
 - a. A stable α -helix unit with 12 or more amino acids in length and
 - 15 b. An hydrophilic, acidic unit with 12 or more amino acids in length which could be a beta-form, a beta-turn, and unordered forms.
2. The pI of a hypersensitive response domain should be acidic and, in general, below 5.
- 20 3. The minimal size of an HR domain is from about 28 to 40 AA residues.

Putative HR domains have been identified to fit the criteria by computer analysis among harpin protein family (Table 5).

09079249.064204
T02T90"84267860

Table 5 - Predication of Hypersensitive Response Domains Among Harpin Proteins

HR domain	Isolated Source	Predicted region*	pI	Structure
HrpN _{Ea} -1	<i>E. amylovora</i>	43-70	3.09	A + H
HrpN _{Ea} -2	<i>E. amylovora</i>	140-176	3.17	A + H
HrpN _{Ech} -1	<i>E. chrysanthemi</i>	78-118	5.25	A + H
HrpN _{Ech} -2	<i>E. chrysanthemi</i>	256-295	4.62	A + H
HrpN _{Ecc} -1	<i>E. carotovora</i>	25-63	4.06	A + H
HrpN _{Ecc} -2	<i>E. carotovora</i>	101-140	3.00	A + H
HrpW _{Pss} -1	<i>P. syringae</i>	52-96	4.32	A + H
HrpW _{Ea} -1	<i>E. amylovora</i>	10-59	4.53	A + H
HrpZ _{Pss} -1	<i>P. syringae</i>	97-132	3.68	A + H
HrpZ _{Pss} -2	<i>P. syringae</i>	153-189	3.67	A + H
HrpZ _{Pss} -3	<i>P. syringae</i>	271-308	3.95	A + H
PopA1 _{Rs} -1	<i>R. solanacearum</i>	92-125	3.75	A + H
PopA1 _{Rs} -2	<i>R. solanacearum</i>	206-260	3.62	A + H

5 *Amino acid residue position

Example 10 - Hypersensitive Response Activity of Select Synthesized Polypeptides

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Polypeptides were produced by expression in either *E. coli* or by chemical synthesis. Based on prediction of solubility and stability of a particular peptide, in some cases, a broader region of AA residues in addition to the essential units were also synthesized to increase solubility of the peptides. The identification of

15

HR domains among four subfamilies of harpin protein demonstrated this (Table 6).

09079249 "061204
T02T90" 84267850

Table 6 - Hypersensitive Response Activity of Select Synthesized Polypeptides

HR domain	Isolated Source	Synthesized region	pI	Source	HR activity
HrpN _{Ea} -1	<i>E. amylovora</i>	43-70	3.09	Chemical Synthesized	+ < 5 µg/ml
HrpN _{Ea} -2	<i>E. amylovora</i>	140-176	3.17	Chemical Synthesized	+ < 5 µg/ml
HrpW _{Ea} -2	<i>E. amylovora</i>	10-59	4.53	E.coli expressed	+ < 5 µg/ml
HrpZ _{Pss} -1	<i>P. syringae</i>	97-132	3.68	Chemical Synthesized	+ < 20 µg/ml
HrpZ _{Pss} -1	<i>P. syringae</i>	153-189	3.69	E.coli expressed	+ < 5 µg/ml
PopA1 _{Rs} -1	<i>R. solanacearum</i>	92-125	3.75	Chemical Synthesized	+ < 5 µg/ml
PopA1 _{Rs} -2	<i>R. solanacearum</i>	206-260	3.62	E.coli expressed	+ < 5 µg/ml

5 Example 11 - Construction of Hypersensitive Response Domains in a Protein Expression Cassette

Polypeptides with a harpin protein hypersensitive response domain were expressed in *E. coli*. PCR was used to amplify desired areas of genes encoding harpin proteins and cloned into an expression vector, e.g. pET28a. A pair of PCR primers with unique flanking sequences were designed to create a universal expression cassette, as shown in Figure 1, for expression of a fragment of harpin protein. Each amplified DNA fragment has a protein translation start codon of ATG in a restriction enzyme Nde I site which might add an extra amino acid of methionine into a polypeptide. Each amplified DNA fragment has a protein translation stop codon of TAA. Each amplified fragment contained two restriction enzyme sites of EcoR V and Sma I, which gave 4 extra in-frame amino acids expressed as Pro-Gly at the N-terminal and Asp-Ile at the C-terminal, respectively. Those two sites are essential to allow two or more expression cassettes to be linked in a specific order and in frame with a minimum number of amino acids being introduced. Cassette A was first digested by EcoR V, ligated to cassette B, and digested with Sma I to produce a new expression cassette C which coupled the two fragments together with two extra amino acids (i.e. Asp-Gly), which are common amino acids in hypersensitive response domains. The newly formed cassette C still contained the same 5' and 3' flanking sequences as original cassettes A and B and maintained the ability to be

coupled by another cassette. Bgl II and Bam HI sites in the cassette permit the cassette to be linked in frame into a concatomer with a correct orientation. The strategy is that digestion of DNA with Bgl II and Bam HI results in compatible ends that would be ligated with each other but could not be cut by either enzymes after ligation. For example, a DNA fragment encoding a hypersensitive response domain in a cassette could be digested by restriction enzymes of Bgl II and Bam HI separately, digested DNA fragments could be ligated in a ligation solution also including both Bgl II and Bam HI enzymes, any ligated ends with Bgl II or Bam HI sites could be digested by the enzymes, and only those ligated sites between Bgl II and Bam HI could remain.

Example 12 - Building Blocks for Creating Superharpins that have Higher Biological Efficacy

Hypersensitive response domains were identified and isolated from several harpin proteins. With the combination of those HR domains, new polypeptides (i.e. superharpins) that have higher HR potency and have enhanced ability to induce disease resistance, impart insect resistance, enhance growth, and achieve environmental stress tolerance. Superharpins could be one HR domain repeat units (concatomer), different combinations of HR domains, and/or biologically active domains from other elicitors. Part of the domains from different harpin proteins and other elicitors were constructed into the universal expression cassette as shown on Example 11 and designated as superharpin building blocks. Table 7 lists some superharpin building blocks which were expressed in pET-28a(+) vector with a His-tag sequence at their N-terminal.

Table 7 - Superharpin Building Blocks including pET-28a(+) his-tag Leader Sequence

Domain Sequence	Source	MW (kDa)	#a.a.	pI	Soluble	(Structurally) Heat Stable
A	PopA70-146	10.69	104	6.48	Yes	Yes
(N _N)	HrpNEa40-80	6.754	68	6.78	N/A	N/A
(N _N) ₂	Dimer of HrpNEa40-80	10.84	111	6.13	N/A	N/A
(N _N) ₃	Triplemer of HrpNEa40-80	14.93	154	5.63	N/A	N/A
(N _N) ₄	Tetramer of HrpNEa40-80	19.01	197	4.95	N/A	N/A
(N _C)	HrpNEa140-180	7.224	68	5.01	Yes	Yes
(N _C) ₂	Dimer of HrpNEa140-180	11.78	111	3.98	Yes	Yes
(N _C) ₃	Triplemer of HrpNEa140-180	16.34	154	3.72	Yes	Yes
(N _C) ₄	Tetramer of HrpNEa140-180	20.89	197	3.58	Yes	Yes
(N _C) ₁₀	Cancatomer (10 repeating units of HrpNEa140-180	48.23	455	3.28	N/A	N/A
(N _C) ₁₆	Cancatomer (16 repeating units of HrpNEa140-180	75.57	713	3.18	N/A	N/A
W	HrpWEa10-59	7.986	77	6.48	N/A	N/A
Z _N	HrpZ90-150	8.087	78	5.38	Yes	Yes
Z ₂₆₆₋₃₀₈	HrpZ266-308	7.029	70	6.40	Yes	Yes
his-tag leader seq.		2.045	19	11.04		

5

Example 13 - Superharpins with Stacked HR Domains and their Biological Activities

There are numerous polypeptides could be generated with different combinations of HR domains or by stacking HR domains and repeating units in order. Selective combination or stacking of HR domains isolated from harpin proteins or other elicitors can be designed to achieve a targeted disease resistance spectrum. See Table 8 for superharpins prepared by stacking of HR building blocks listed on Table 7. All three listed superharpins (i.e. SH-1, SH-2, SH-3) were constructed into a pET28(a) vector and expressed in *E. coli*. Recombinant proteins were partially purified and quantified by SDS-PAGE with purified Harpin N protein as a quantitative standard.

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Table 8 - Properties of Superharpins

Protein	Domain Sequence	MW (kDa)	# a.a.	pI	Soluble	Heat Stable
SH-1	*W(N _N) ₄ A(N _C) ₄ Z ₂₆₆₋₃₀₈	54.955	545	3.69	Yes	Yes
SH-2	*W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈	52.341	519	3.54	Yes	Yes
SH-3	*W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈ A	60.375	598	3.67	Yes	Yes
HrpNEa	HrpN from <i>E.amylovora</i>	39.697	403	4.42	Yes	Yes

- 5 Bioassays for hypersensitive response on tobacco leaves (HR), percentage of TMV reduction on tobacco leaves, and plant growth enhancement with tomato showed that superharpins had higher (up to 2 to 10 fold greater) HR potency compared with HrpN from *E. amylovora*. This also demonstrated that superharpins have better performance on % TMV reduction and plant growth enhancement assay.
- 10 See Table 9.

Table 9 - Biological Activities of Superharpins

Protein	Domain Sequence	Elicit HR (~µg/ml)	% TMV reduction on tobacco		% Plant Growth Enhancement	
			10 µg/ml	1 µg/ml	10 µg/ml	1 µg/ml
SH-1	W(N _N) ₄ A(N _C) ₄ Z ₂₆₆₋₃₀₈	0.66	83	79	7.49	9.83
SH-2	W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈	0.13	84	60	11.05	7.30
SH-3	W(N _N) ₄ Z _N (N _C) ₄ Z ₂₆₆₋₃₀₈ A	0.15	77	55	11.07	10.00
HrpNEa	HrpN from <i>E.amylovora</i>	1-3	55	10	11.68	N/A

- 15 Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.